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14. ABSTRACT

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The top down approach of iteratively removing non-essential genes from M. mycoides syn1.0 continues. Genes or sets of genes are deleted from previous top down constructs while the genomes are parked in yeast cells. Then the reduced genomes are isolated from yeast cells and transplanted to determine if the deletion is viable or has an altered phenotype. This effort is more about directing the bottom up approach by identifying potential synthetic lethals than about eventually reaching a fully minimized genome by iterative gene deletion. Currently we are working on our 20th iteration of this process. The smallest genome the top down effort has made to date has 743 kb and is missing 240 protein coding genes. This deletion mutant grows normally.

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J. Craig Venter™
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Construction of a Bacterial Cell that Contains Only the Set of Essential Genes Necessary to Impart Life

Report Title:	R&D Status Report (Quarterly)
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Reporting Period:	May 17, 2014 to August 16, 2014
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Abstract

We now have a viable reduced genome design (RGD) cell that is approximately 597 kb. This is a genome in which seven of the eight genome segments are minimized. It grows at less than half the rate of wild type *Mycoplasma mycoides*. This genome is comprised of minimized segments 1 and 4 (from the RGD2 design), minimized segments 2, 3*, 6, 7, and 8 (from the RGD1 design), and a wild-type, non-minimized segment 5. The "3*" designation denotes that this segment was derived from the RGD1 design, with three genes involved in glycerol metabolism added back into the sequence. The key issue that we must resolve to produce a fully minimized RGD is that in RGD2 segment 5 there are one or more instances of what we call synthetic lethal deletions that we have to identify and add back to the genome. A synthetic lethal is the result of deleting both members of a pair of genes that both encode the same essential function. Individually, either member of the pair can be deleted; however a genome with both genes omitted will not support life. We are currently working to identify the remaining synthetic lethals so that RGD2 segment 5 can be redesigned and synthesized.

The top down approach of iteratively removing non-essential genes from *M. mycoides* syn1.0 continues. Genes or sets of genes are deleted from previous top down constructs while the genomes are parked in yeast cells. Then the reduced genomes are isolated from yeast cells and transplanted to determine if the deletion is viable or has an altered phenotype. This effort is more about directing the bottom up approach by identifying potential synthetic lethals than about eventually reaching a fully minimized genome by iterative gene deletion. Currently we are working on our 20th iteration of this process. The smallest genome the top down effort has made to date has 743 kb and is missing 240 protein coding genes. This deletion mutant grows normally.

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The effort to modularize the genome is progressing on three fronts. A 30 gene tRNA module was previously constructed and inserted into the *M. mycoides* syn1.0 genome. We are now determining if that genome will remain viable as each of the remaining 12 natural tRNA clusters are removed such that the synthetic genes in the module must support the tRNA needs of the cell. We have designed modules for other functional groups including arginine hydrolysis, glycerol metabolism, and amino acyl tRNA synthetases. Finally, in an effort to determine the functions of genes of unknown function that are similar to genes from other bacteria that encode biochemically characterized proteins, we are swapping native uncharacterized genes for characterized genes from *Bacillus subtilis*, that is presented in a constitutive expression module. If the *B. subtilis* gene containing *M. mycoides* mutant is viable than the function of the conserved hypothetical gene is the same as the input *B. subtilis* gene.

Table of Contents:

Summary

The goal of the project is to create a cell that contains only the set of genes that are essential for life under ideal laboratory conditions. We are working to minimize *Mycoplasma mycoides* JCVI-syn1.0 (the synthetic version of *Mycoplasma mycoides* subsp *capri*) using two approaches:

- Top Down: remove genes and clusters of genes one (or a few) at a time, proceeding only if the reduced strain is viable, with a reasonable growth rate
    - We previously reported the results of a transposon study was conducted and allowed us to categorize genes as Essential (E), Non-essential (N), or Impaired (I)
        - This categorization scheme has been the basis of most of our subsequent work
    - The *M. mycoides* genome has been reduced to 743 kb using the Tandem Repeat Endonuclease Cleavage (TREC) strategy
- Bottom Up: design a reduced genome based on our best Tn5 gene disruption and deletion data (RGD), and synthesize it
    - Synthesis from oligonucleotides
        - All 1/8th genome molecules have been tested and found to be viable
        - Viable genomes containing multiple RGD segments have been constructed
        - Two genomes with seven minimized segments have been found to be viable:
            - RGD1 segments 2, 3*,6, 7, 8, RGD2 segments 1, 4, and Wild-type 5; 597 kb
            - RGD1 segments 2, 6, 7, 8, RGD2 segments 1, 3, 4, and Wild-type 5; 604 kb
        - We believe that even after the adding several genes back to the RGD1 version of segment 5 to make the RGD2 segment 5 there is still a remaining synthetic lethal gene or genes. This thus has far prevented the transplantation of a viable minimal cell. We are working to identify these genes
            - One of our hypotheses about the remaining synthetic lethal(s) is that it is one or more genes involved in synthesis of the membrane lipid cardiolipin. While we are looking to find the gene, we have an alternative approach to try. We will attempt to add cardiolipin to the growth medium. To our knowledge this has never been tried with any mycoplasma media. Cardiolipin solubility may

be the issue. We plan to investigate *M. mycoides* growth in SP4 medium supplemented with cardiolipin and also to transplant full RGD2 versions into this media.

tRNA Modularization: The initial modularization experiments are progressing. A 5.3 kb tRNA module containing the 30 tRNA genes plus the necessary promoters and terminators was constructed and sequence verified. The module was inserted into the genome in place of the largest natural cluster of tRNAs and found to be viable. In Q2 we tested the effects of removing the remaining 12 tRNA clusters from the cell individually so that substitution of each of the 30 natural tRNAs with the ones encoded in the module can be tested. All substitutions proved to be viable. Now we are resynthesizing the genome with only the 30 tRNA module.

Interspecies modules to characterize unknown genes: We observed that a gene annotated as a conserved hypothetic protein had weak similarity to biochemically characterized pseuduridinte methyltransferase genes (*rlmH*) in other bacteria. We replaced essential gene MMYC_0361 with the *rlmH* gene from *Bacillus subtilis. Mycoplasma mycoides* containing the *B. subtilis rlmH* was viable. This tells us the function of a previously unknown essential gene. Efforts are now underway to do this with other *M. mycoides* essential genes of unknown function that have weak similarity to characterized genes in other species.

Introduction

The goal of this research project is to build a minimal bacterial cell that contains only the genes that are required for life in ideal laboratory conditions. The pursuit of a minimized cell is critical to the advancement of biology, both as a pathway for understanding the basic requirements for replication and as a chassis for creating an optimized platform for any number of possible applications.

We previously reported that the *Mycoplasma mycoides* JCVI-syn1.0 genome was successfully reduced from 1078 kb to 779 kb; however, while the 779 kb genome was viable, the growth rate was far too slow to allow follow up experiments at an acceptable pace. Using the N, E, I gene categories, the genome has been reduced to 743 kb, but with a normal doubling time.

As reported previously, all 8 of the $1/8^{th}$ RGD segments tested have proven to be individually viable when in the complementing $7/8^{th}$ background. We now have several genomes that contain up to 5 minimized segments, one genome that has 6.5 minimized segments, and two genomes that have seven minimized segments.

A preliminary tRNA module was designed, constructed, and introduced into the *M. mycoides* syn1.0 genome and found to be viable. Synthesis of segments with the natural tRNA loci removed is in progress.

Throughout our genome minimization process we have been establishing rules for genome remodeling. As a precursor to grand scale modularization of the genome, we have synthesized a fully modularized version of RGD1 segment 2. In the next reporting period we will report on whether genomes made with this radically redesigned segment are viable. If it is then we have a working set of rules for large scale genome remodeling.

Methods, Assumptions and Procedures

TOP DOWN APPROACH

The plan here was to start with the full size 1078 kb *M. mycoides* JCVI-syn1.0 synthetic genome. We have continued to use the TREC strategy to make iterative deletions in the mycoplasma genome. Targeting the N category genes and clusters is proving to be effective (further discussed

in the Results and Discussion section). We have made a series of strains that are progressively reduced with little to no reduction in growth rate.

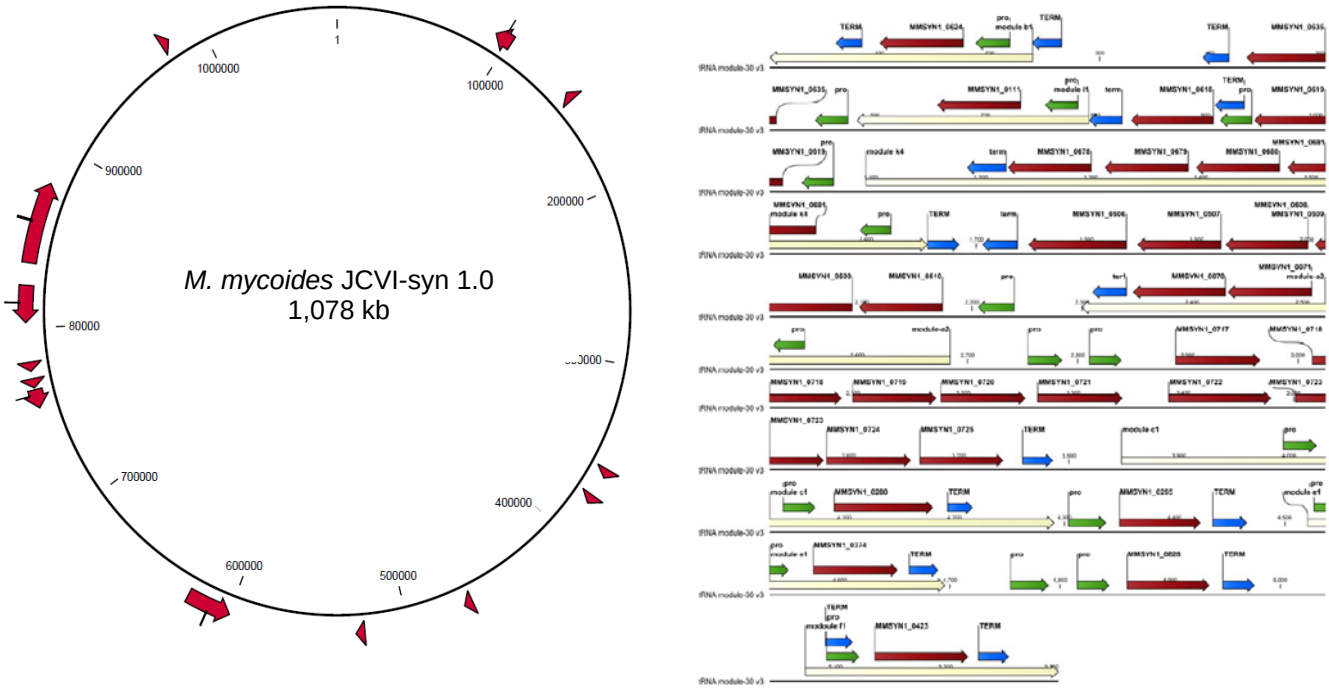
BOTTOM UP APPROACH

Synthesis from oligonucleotides: Two genome designs were completed using the N, E, I gene classification system. All individual segments have been found to support cellular life. We have continued testing combinations of reduced genome segments. We now have several genomes that contain up to seven RGD segments.

MODULARIZATION

To test gene modularization, we have organized the 30 tRNA genes of *M. mycoides* into a single contiguous module. The module contains the coding regions, as well as the promoters and terminators needed for regulation. The tRNA genes are naturally distributed around the genome in 13 loci.

Figure 1



(a) Natural distribution of tRNA genes in M. mycoides. *The tRNA gene clusters have been enlarged in Fig.1(a) to show the direction of transcription. The M. mycoides JCVI-syn1.0 genome has 8 single tRNA genes and 5 clusters of 2 to 9 genes, for a total of 30.*
(b) tRNA module design. *The 30 tRNA genes have been relocated into a single module. (Green*

arrows represent promoters. Red arrows show tRNA genes. Blue arrows are terminators.)

Each of the 13 loci was synthesized by PCR using syn1.0 as the template, cloned in *E. coli* and then joined together into a single cassette with appropriate yeast markers. The cassette was inserted into syn1.0 to replace the largest cluster of 9 tRNAs at 10 o'clock on the genome map. The resulting genome is viable after transplantation. We have now made 12 other genomes in which each tRNA cluster was replaced with the synthetic 30 tRNA cluster. Each of these proved to be viable. This tells us that the tRNA module can replace all of the native tRNA genes, Based on this finding, we will now synthesize segments with the 12 other tRNA loci around the genome removed from the design.

GENOME COMPLIMENTATION

We have produced partially minimized strains with undesirable phenotypes such as slow growth or no capacity for transposon mutagenesis. We have begun initial experiments aimed at enabling genetic complementation to restore desirable phenotypes to deleted strains, for example, RGD. A system capable of quickly adding deleted genes back into a genome would be a powerful tool to help de-convolute growth-retarding synthetic effects.

To do this we make Tn5 transposons that either contain the genes we expect to correct the undesirable phenotype and the transpose the mutant. If the undesirable phenotype is lost, we have identified the gene that corrects it and now have expanded knowledge of minimal cell gene function. If we do not have any ideas about what causes the undesirable phenotype, we construct Tn5 transposons containing 10 kb random fragments of the wild type M. mycoides. After transposition we look for cells without the phenotype and then use DNA sequencing to determine what genes can correct the phenotype. We have now used these complementation approaches to determine what genes are responsible for slow growth in some strains.

Results and Discussion

MODULARIZATION

tRNAs: As reported in Q1, we have constructed a synthetic tRNA module that encodes all 30 tRNAs, transcriptional promoters and terminators as a single 5.3 kb cassette. We substituted the cassette for the 9 tRNA cluster of tRNAs in *M. mycoides*, and that cell grew normally. This was an important first step. We now have a cell with a single copy of 9 tRNAs and two copies of the other 22. In Q2 we tested whether cell made previously would remain viable if individually removed the other 12 clusters of tRNAs. To date all 12 tRNA cluster deletions yielded viable *M. mycoides*; although we were surprised that some of these grow slower than the wild type cell.

We are now building RGD (reduced genome design) minimal cell modules with the tRNA modules replaced by a transcriptional terminator (we do not want unintended transcription from the genes flanking the tRNA site disrupting the cell).

Module swap to characterize genes of unknown function: In another aspect of the modularization effort, we have replaced an essential gene annotated in *M. mycoides* as a conserved hypothetical protein with the *B. subtilis rlmH* gene, which produces a biochemically characterized pseudouridine methyltransferase enzyme.

Figure 2

A Swapping characterized gene expression modules built from other bacterial species into M. mycoides to characterize unknown genes.

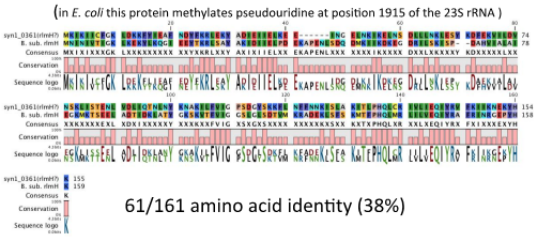
- Dig deeper into the list of BLAST hits to look for something plausible.
- Test possible functional assignments of a gene by replacing it with a well characterized gene from another species.

A test case – MMSYN1_0361 (essential by Tn5)

Annotation Source	Function annotated
Chuck's pipe dream	Ribosomal RNA large subunit methyltransferase H
Original annotation	conserved hypothetical protein
Production pipeline	putative rRNA large subunit m3Psi methyltransferase RlmH
CHAR pipeline	putative rRNA large subunit m3Psi methyltransferase RlmH
SGI pipeline	Ribosomal RNA large subunit methyltransferase H

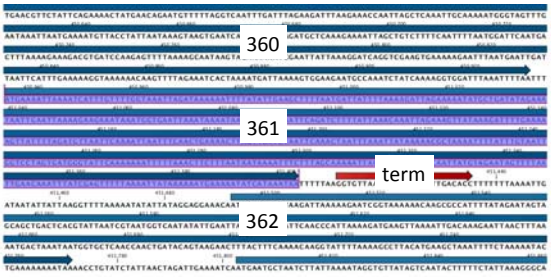
B Alignment of the product of syn1.0 gene 361 ("conserved hypothetical protein") with *B. subtilis* rlmH gene product

(in *E. coli* this protein methylates pseudouridine at position 1915 of the 23S rRNA)



61/161 amino acid identity (38%)

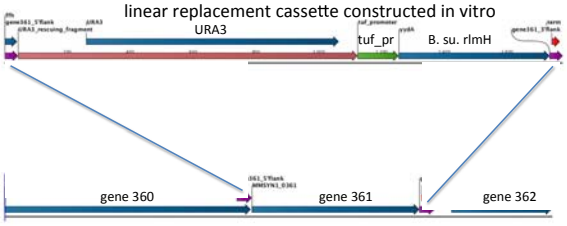
C syn1.0 gene 361 (rlmH?) context



Genes 359-366 are keepers. 364 is a very strong "i" and the others are all "e".

D Gene replacement by recombination in yeast

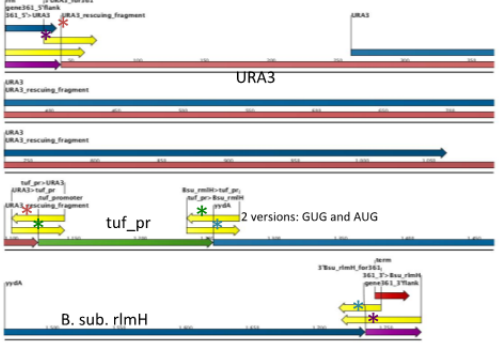
linear replacement cassette constructed in vitro



syn1.0 genome in yeast

- Introduce cassette into URA3- yeast by lithium acetate transformation
- Select yeast on Uracil minus plates
- Screen by PCR using primers in genes 360 and 362
- Pool a number of colonies and transplant into *M. capricolum*

E Cassette to replace gene 361 with *Bacillus subtilis rlmH*



F Positive clones for each construction were pooled & transplanted

- Positive yeast clones were pooled and grown up in liquid.
- Plugs were prepared.
- Bogumil transplanted 2 yeast plugs into *M. capricolum*
- All three constructs gave transplants with colonies similar in size to syn1.0

Bsu_rlmH (GUG)	11 colonies
Bsu_rlmH (AUG)	13 colonies
MMSYN1_0361	21 colonies

- It appears we have replaced the essential gene MMSYN1_0361 with Bsu_rlmH and the mycoplasma is fully viable.
- Since the *B. subtilis* gene is known to encode RNA large subunit methyltransferase H, we infer that MMSYN1_0361 has the same function.
- There are a couple of controls I plan to do:
  1) Screen for an internal fragment of 361
  2) Replace 361 by just URA3. This should be dead.
- We should develop a list of candidate genes from the RGD for applying this approach to confirm gene function.

Fig. 2. Swapping characterized gene expression modules built from other bacterial species into *M. mycoides* to characterize unknown genes. (A) As an example of this approach, we swapped a synthetic expression module for B. subtilis psuedouridine methyltransferase gene rlmH with an essential gene of unknown function in M. mycoides. This characterized rlmH gene is slightly similar to the *M. mycoides* essential gene MMYC_0361. (B) This gene was originally annotated in *M. mycoides* as a conserved hypothetical protein. The encoded protein is 38% identical to a characterized gene in *B. subtilis* that encodes the ribosomal RNA large subunit pseudouridine methyltransferase H. (C) The locale of MMYC_0361 in the *M. mycoides* chromosome. (D) To swap the *B, subtilis* gene for the *M. mycoides* gene in yeast, a cassette containing a URA3 marker and the *B. subtilis rlmH* gene was constructed with the rlmH gene behind the *M. mycoides tuf* promoter, which is a strong promoter. It was exchanged into the YCp and that was transplanted into *M. capricolum*. (E) PCRs were done using primers at the asterisks to confirm that the resulting transplants had the desired genes. (F) Analysis confirmed that *B. subtilis rlmH* could replace the *M. mycoides* MMYC_0361 gene and that the MMYC_0361 gene likely encodes ribosomal RNA large subunit methyltransferase H. We envision using this method to evaluate the function of many of the unknown genes in the minimal cell.

Facinating Unexpected Finding about the Minimal Cell Phenotype not related to any Specific Milestone

In our efforts to characterize partially minimized M. mycoides strains we performed electron microscopy. The scanning electron micrographs below are of different synthetic mycoplasma cells grown in liquid culture. Panels A & B show the in essence wild type cells (*Mycoplasma mycoides* JCVI syn1.0). The bar shows 1 micron, so the cells are about 500-700 nm in diameter. The cells shown in panels C & D have genomes that have the RGD segment 6 and the other seven segments are wild type. At first we thought we could explain these enormous cells that are ~1000X greater in volume than wild type cells by the lack of the genes encoding the cell division protein FtsZ and FtsA (it is also missing 78 other non-essential genes). Later we saw that a top down mutant that was essentially only missing the *ftsZ* and *ftsA* genes was the same diameter as wild type cells. We are mystified as to what are all the gene deletions that result in this phenotype. If the loss of cell division proteins FtsZ and FtsA had been sufficient to cause it, the phenotype would fit with some existing hypotheses about the evolution of cell division. We will continue to pursue this mystery and also examine these giant cells to determine if they offer any useful opportunities for designer microbes.

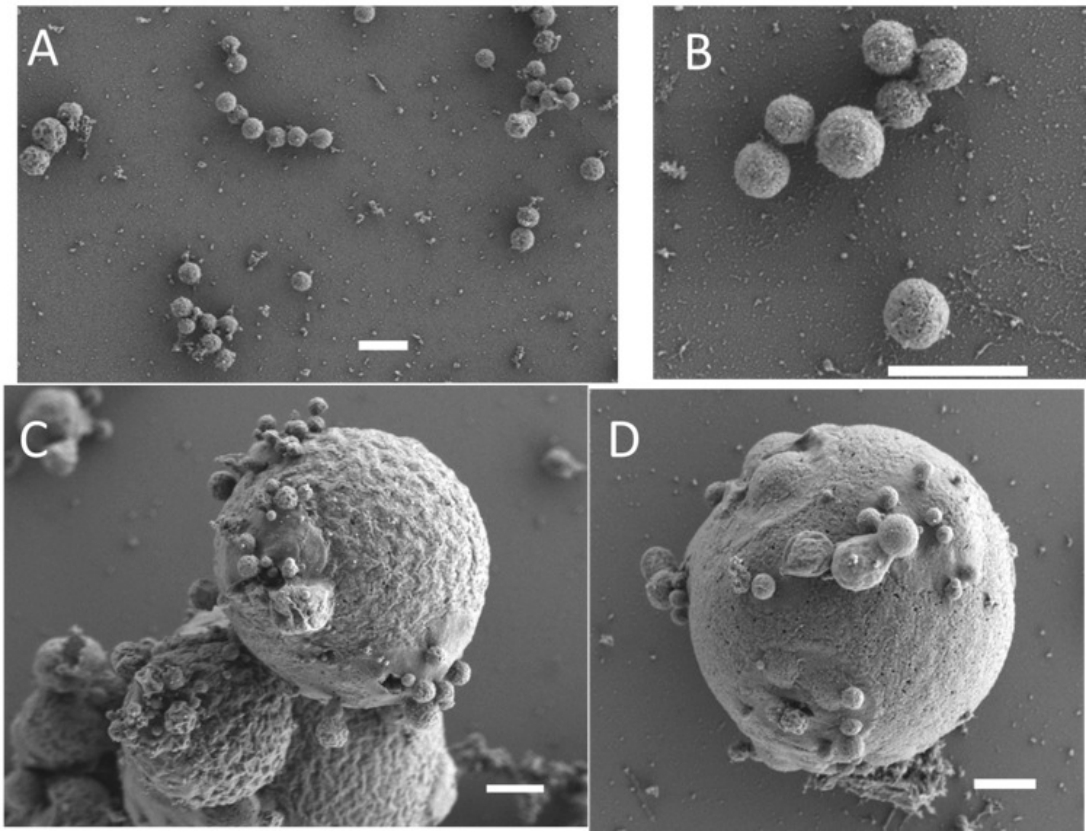


Figure 3. Electron micrographs of wild type (JCVI syn1.0) cells in panels A and B, and a partially minimized *M. mycoides* containing a reduced segment 6 and wild type versions of the other 7 segments in panels c and D. The bar is 1.0 micron.

TOP DOWN APPROACH

Iterative deletions using the TREC based approach are making steady progress toward a minimal genome. A table outlining the progress to date is shown below. Since the last reporting period, strain D17, D18 and D19 have been tested and found to be viable with a good qualitative growth rate (quantitative growth rate evaluation has not been performed).

Table 1

Strains	DT(min)	Genome Size (bp)	# of Genes Deleted
syn1.0	64	1,078,809	0
syn1.0D6 RE		1,062,183	17
DISs		1,048,690	31
D1		979,083	68
D2		969,069	74
D3		944,159	90
D4		931,710	97
D5		923,647	102
D6	67	908,931	108
D7		877,942	135

D8		866,271	155
D9	64	844,265	173
D10	65	828,901	181
D11		816,807	194
D12		805,506	201
D13		794,666	200
D14		784,762	207
D15		775,131	216
D16		763,995	224
D17		757,001	230
D18		749,520	235
D19		~743,000	240
D20*			

*In progress

Sequential deletion of genes and clusters of genes is not the way that we ultimately expect to pursue a minimal genome design. While we are making steady progress in this approach, the greater outcome of performing the top down deletions is the generation of information regarding unanticipated interactions between elements of the genome that are difficult or impossible to identify using the Bottom Up approach. De-convolution of synthetic lethal effects will be difficult work, which we hope to delay as long as possible with information gained from the top down approach.

BOTTOM UP APPROACH

Work this quarter has centered on identification of the synthetic lethal genes, and design of a new genome that encodes the essential functions. Not only do we want to identify the problematic genes, we also want to evaluate different methods for making the identification. We are going about this process using multiple approaches:

- From both our own analyses and from literature data on related mycoplasmas, we have come to realize that glycerol metabolism is essential and that we had deleted all the genes capable of importing glycerol into the minimal cell. It was the hypothesis of the *M. mycoides* research community that if the glycerol metabolism genes could be deleted, the resulting cell would be non-pathogenic and possibly usable as a live attenuated vaccine. This is because one of the byproducts of glycerol metabolism in *M. mycoides* is hydrogen peroxide, which is a virulence factor (other subspecies of *M. mycoides* are a major cattle pathogen in sub-Saharan Africa and there is no good vaccine). Our work has dashed that hypothesis. The operon comprised of glycerol kinase, glycerol oxidase and the glycerol uptake facilitator will be put back in RGD segment 3. Alternatively we could have put back glycerophosphodiester phosphodiesterase into segment 2 and a glycerol-3-phosphate transporter protein into segment 5. Similarly, we are re-examining the genes we have removed that have annotations in hopes of realizing based on our knowledge of biology that we have removed genes known to encode essential functions.
- We have done tn5 bombardment of on *M. mycoides* mutants that were 7/8^{ths} wild type and 1/8th RGD to look for genes that were not essential in the fully wild type *M. mycoides*, but could not be hit with transposons when sets of genes had been removed. This pointed to a series of potential problem genes that we are considering putting back in the RGD. We were expecting only one or two genes to become essential in each 7/8th wild type 1/8th RGD; however we found many more than that, which has made analysis more complicated (we did see that glycerol metabolism genes 217-219 became essential when the aforementioned genes in segments 2 or 5 were missing).
- In still another approach using a fast growing RGD24678 clone, we used TREC to one at a time systematically delete all the clusters of genes that we originally thought were not essential in

segments 1 and the part of segment 3 that was RGD in RGD23*45678 Clone 59. Once again, the data point to more than a single gene or group of genes to be restored to the RGD. This could be because there are multiple synthetic lethals to be repaired in segments 1 and 3 or because of other problems not related to the synthetic lethals.

- Unexpectedly, we found that cells containing RGD4 could not be transformed with tn5. By doing complementation studies in RGD5 cells we have determined that at least one of the genes in a cluster of seven genes omitted from RGD4 is necessary for tn5 transformation.
- Based on our analyses using the methods described above, we redesigned the RGD to make RGD2, which will contain 26 genes previously omitted from RGD that we have evidence might be needed for viability (and tn5 transformability). We chose these genes to both generate a viable cell and a cell that grows at the same rate as wild type *M. mycoides*. We realize some of these added genes are probably not essential, but the priority is to generate a viable near minimal cell with each 1/8th genome segment almost fully minimized. This RGD2 design, at 574,572 kb will still be smaller than the genome of *Mycoplasma genitalium,* which at 579,508 bp has the smallest genome of any organism that can be grown in axenic culture. Later we can do an additional tn5 bombardment of the RGD2 genome to identify any genes that can still be deleted from the genome either by TREC or re-synthesis.

Conclusions

Tasks from the Statement of Work:

Task 1: **Complete a detailed global Tn5 transposon mutagenesis insertion map.**
The Tn5 transposon insertion map was submitted with the initial quarterly report.

Due: Month 6; Status - complete

Task 2: **Delete up to 27 large gene clusters**
Using top down approaches we have reduced the genome size of *M. mycoides* JCVI-syn1.0 from 1079 kb to 764kb through the deletion of some >35 clusters, representing a ~30% reduction. Using the bottom up approach we have a genome with ~85% of the non-essential genes removed in a ~590 kb genome, which makes it the second smallest cell known to grow I pure culture.

Due: Month 12; Status – complete

Task 3: **Construct a preliminary modular map of the genome**
The design of a modular map of the genome is complete was presented in the May 2012 quarterly report

Due: Month 12; Status – complete

Task 4: **Make new transposon insertion map. Identify non-essential small 2-4 gene clusters. Delete small clusters.**
A transposon study was performed and previously reported. A table showing the resulting N/E/I categorization system was presented in May 2013 (Deliverable 1). In Q2 we performed tn5 bombardment on our individual RGD cassettes as well as some of the multiple cassette strains in order to identify synthetic lethals and identify remaining non-essential genes.

Once the RGD is tested and viable, we will use Tn5 mutagenesis to discover if the classifications of any of the remaining genes have changed and to determine and report which additional genes might be removed (Deliverable 2).

Task 5: Identify non-essential single genes. Delete individual genes.
This is dependent upon the creation of a minimal genome. *We are very close to having a viable RGD genome*. This will likely be termed RGD3, and will be analyzed using transposon mutagenesis for any remaining non-essential genes.

Task 6: Complete the removal of non-essential genes and sequences and characterize the final minimal cell product.
Similar to Task 5, work on this task will commence once a viable, eight segment RGD is transplanted.

Task 7: Refine the modular map. Construction and testing of a module as a proof of principle.
We have now shown that existing 30 tRNA genes can be combined into one module and that the tRNA genes in the module can substitute for each of the natural tRNA genes. Shortly we will have a RGD cell in which only the 30 tRNAs in the module are a source of tRHAs.

We have designed modules for glycerol metabolism, arginine hydrolysis (which would enable the cell to use arginine hydrolysis as an energy source), and amino acyl tRNA synthetases. These will be built an tested in a manner similar to what we have done for the tRNAs.

As a precursor to whole genome modularization, we have constructed (although not yet tested) a whole RGD genome segment that was completedly redesigned into functional modules. This will be built into a genome and tested in the next quarter.

Planned Activities for the Next Reporting Period

1. Continue the Top Down minimization of our synthetic genome (near term) and conduct a new transposon study on the minimized genome.
2. Now that we have a cell whose genome is comprised of 7 reduced segments plus a wild type segment 5, we will determine what genes are missing from segment 5. The RGD3 segment will be constructed and tested.
3. Continue with verification and testing of the tRNA gene module.
4. Continue swapping expression modules from characterized *B. subtilis* genes with similar but as yet uncharacterized essential genes from *M. mycoides* to determine the functions of the unknown genes.
5. Test genomes containing the fully modularized genome segment 2.
6. Write manuscript(s) describing the RGD cell and early efforts at modularization.

Program Financial Status

In Process & Completed Tasks	Planned Expend	Actual Expend (Cumulative to Date)	% Budget Completion	At Completion	Latest Revised Estimate	Remarks
Task 1	305,646.00	305,646.00	100%	305,646.00	305,646.00	Completed
Task 2	826,256.00	798,351.00	97%	798,351.00	826,256.00	Completed
Task 3	43,487.00	43,487.00	100%	43,487.00	43,487.00	Completed
Task 4	1,100,000.00	1,084,923.00	99%	N/A	1,100,000.00	In Progress
Task 5	243,977.33	109,679.26	45%	N/A	243,977.33	In Progress
Task 6	243,977.33	109,679.26	45%	N/A	243,977.33	In Progress
Task 7	243,977.33	109,679.26	45%	N/A	243,977.33	In Progress
Cumulative	3,007,321.00	2,561,444.79	85%	N/A	3,007,321.00	N/A

There is no management reserve or unallocated resources. The financial data presented is current through Dec. 2013.

Based on the currently authorized work:

- Is current funding sufficient for the current fiscal year? Yes
- Have you included in the report narrative any explanation of the above data and are they cross-referenced? Not applicable; current funding is sufficient for the current fiscal year.